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A vertebrate fatty acid desaturase with Δ5 and Δ6 activities

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Abbreviations: PUFA, polyunsaturated fatty acid(s); FAME, fatty acid methyl ester(s)

Data deposition: Genbank accession no. AF309556, zebrafish Δ5/Δ6 desaturase cDNA

Abstract

Δ5 and Δ6 fatty acid desaturases are critical enzymes in the pathways for the biosynthesis of the polyunsaturated fatty acids (PUFA) arachidonic, eicosapentanoic and docosahexanoic acids. They are encoded by distinct genes in mammals and in *Caenorhabditis elegans*. This paper describes a cDNA isolated from zebrafish (*Danio rerio*) with high similarity to mammalian Δ6 desaturase genes. The 1590 bp sequence specifies a protein that, in common with other fatty acid desaturases, contains an N-terminal cytochrome b5 domain and three histidine boxes, believed to be involved in catalysis. When the zebrafish cDNA was expressed in *Saccharomyces cerevisae* it conferred the ability to convert linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) to their corresponding Δ6 desaturated products, 18:3n-6 and 18:4n-3. However, in addition, it conferred on the yeast the ability to convert di-homo-γ-linoleic acid (20:3n-6) and eicosatetraenoic acid (20:4n-3) to arachidonic acid (20:4n-6) and eicosapentanoic acid (20:5n-3), respectively, indicating that the zebrafish gene encodes an enzyme having both Δ5 and Δ6 desaturase activity. This is the first report of a functionally characterized desaturase enzyme of fish, and the first report of a fatty acid desaturase in any species with both Δ5 and Δ6 activity. The zebrafish Δ5/Δ6 desaturase may represent a component of a prototypic vertebrate PUFA biosynthesis pathway.

Introduction

Vertebrates lack the Δ12 and Δ15 fatty acid desaturases responsible for converting oleic acid (18:1n-9) into linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3), and are thus unable to biosynthesize polyunsaturated fatty acids (PUFA) *de novo* (1,2). PUFA are, therefore, essential dietary nutrients for vertebrates (1,2). The physiologically active PUFA are arachidonic acid (AA, 20:4n-6), eicosapentanoic acid (EPA, 20:5n-3) and docosahexanoic acid (DHA, 22:6n-3), which are required for optimal health and normal development of vertebrates (3-6). The pathway from 18:2n-6 to AA, and from 18:3n-3 to EPA involves desaturations at the Δ6 and Δ5 positions in the carbon backbone, and an intermediate 2-carbon chain elongation step (7). Production of DHA from EPA requires an additional desaturation and 2-carbon chain elongation, although the mechanism may be more complicated (8). However, there is considerable variation between animal species in their abilities to synthesize the C_{20} and C_{22} PUFA from the plant-derived C_{18} precursors 18:2n-6 and 18:3n-3. Some animals, notably extreme carnivores, have a very limited ability to synthesize C_{20} and C_{22} PUFA, and consequently have a strict requirement for a dietary source of pre-formed C_{20} and C_{22} PUFA (9-12). While humans generally possess the ability to synthesize C_{20} and C_{22} PUFA from linoleic and α-linolenic acids, dietary changes as a consequence of intensification of agriculture have resulted in an increase in the 18:2n-6/18:3n-3 ratio in foods (13). There is considerable evidence that this has had, and continues to have, negative impacts on health and development in affected populations (4,13). The dietary 18:2n-6/18:3n-3 ratio appears to have increased due to a steady decline in dietary n-3 fatty acids over a period of several hundred years, and this has been compounded by an increased dietary intake of 18:2n-6 in recent decades, particularly in western societies (13-16).

Meeting the dietary demands of a burgeoning human population for a correct dietary balance of PUFA, and at levels required for normal health and development, is a major challenge. Clearly, an understanding of the molecular basis of PUFA biosynthesis could underpin efforts to meet this challenge. However, until recently, and although the biochemical pathways involved in PUFA synthesis were described, little was known of the enzymes involved and of the factors affecting their function(s). Some progress has been made recently in characterizing the elongase and

desaturases involved in PUFA synthesis (17). Full length cDNAs for Δ6 desaturases have been isolated from the nematode *Caenorhabditis elegans* (18), rat (19), mouse and human (20). Fatty acid Δ5 desaturase genes have been isolated from *C. elegans* (21,22) and human (23,24).

We have undertaken to study the PUFA synthesis pathway in fish, for two reasons. First, fish are an important source of PUFA, especially of the long chain C_{20} and C_{22} n-3 PUFA that are often deficient in human diets (14-16). Second, there is wide variation between fish species in their ability to synthesize PUFA (25-27). Many freshwater species such as trout, tilapia and carp are able to convert dietary C_{18} precursor fatty acids to AA, EPA and DHA. However, marine species such as turbot and sea bream, which are inherently piscivorous, have very limited abilities to perform these conversions (26-31). Comparison of the genes encoding key elements in the fatty acid desaturation pathway between freshwater and marine species may, therefore, increase knowledge of the molecular components of the pathway itself, and of the molecular genetic basis of phenotypic variation in PUFA biosynthesis.

We have recently demonstrated that zebrafish (*D. rerio*) share the capacity of other freshwater fish species to synthesize C_{20} and C_{22} PUFA from vegetable oil-derived C_{18} dietary precursors (32). Therefore, because Δ6 desaturation is generally considered to be the rate-limiting step in PUFA synthesis (33), we have initially targeted the Δ6 desaturase gene(s) of zebrafish. Here we describe the cloning and functional characterization of a novel fatty acid desaturase gene obtained from zebrafish, which, uniquely among such genes described to-date, has both Δ5 and Δ6 desaturation activities.

Materials and Methods

Isolation of a zebrafish desaturase cDNA and sequence analysis. A zebrafish EST sequence (Genbank accession no. AI497337) was identified that displayed high homology to mammalian Δ5 and Δ6 desaturase genes. cDNA was synthesized from zebrafish liver total RNA using MMLV reverse transcriptase primed by the oligonucleotide, 5'-GATAGCGGCCGCGTTTTTTTTTTTTTTT(AGC)-3'. A portion of this cDNA was then subjected to PCR amplification (Ready-to-Go PCR beads; Amersham Pharmacia Biotech) with the primer described above and an oligonucleotide (5'-ATGGGTGGCGGAGGACAGC-3') predicted from the zebrafish

EST sequence to contain the protein initiation codon. Amplification involved an initial denaturation step at 94 ^{o}C for 1 min, followed by 30 cycles of denaturation at 94 ^{o}C for 30 s, annealing at 57 ^{o}C for 30 s, and extension at 72 ^{o}C for 3 minutes. The products were cloned into the pYES2 plasmid (Invitrogen Ltd., Paisley, U.K.) using standard methods, and nucleotide sequences were determined using a Perkin Elmer ABI-377 DNA sequencer. Deduced amino acid sequences were aligned using ClustalX and sequence phylogenies were predicted using the Neighbour Joining method of Saitou and Nei (34). Confidence in the resulting phylogenetic tree branch topology was measured by bootstrapping the data through 1000 iterations.

Expression of the zebrafish desaturase cDNA. The coding sequence of the zebrafish cDNA was amplified using the forward primer 5'-CCCAAGCTTACTATGGGTGGCGGACAGC-3' and the reverse primer 5'-CCGCTGGAGTTATTTGTTGAGATACGC-3'), containing HindIII and XhoI sites, respectively. The amplified product was ligated into the HindIII and XhoI sites of the pYES yeast expression vector (Invitrogen). The resulting plasmid constuct, pYESZFB10, was transformed into *Saccharomyces cerevisiae* (strain INVSc1) using the EasyComp transformation kit (Invitrogen). Yeast transformed with either the pYES vector or with pYESZFB10 were cultured overnight in 2% raffinose, 0.67% nitrogen base, 1% tergitol type NP-40 and 0.19% uracil dropout media at 30^{0}C. The cultures were then diluted to an OD_{600} of 0.4 and grown until they reached an OD_{600} of 1, after which expression of the transgene was induced by the addition of galactose to 2% w/v . At this point the cultures were supplemented with one of 0.5mM 18:2(n-6), 18:3(n-3), 20:3(n-6) or 20:4(n-3), and then maintained at 30^{0}C in a shaking incubator. Samples were taken for analysis 48 hours following galactose induction.

Fatty acid analysis. Approximately equal amounts of yeast cells were transferred into glass conical test tubes after determination of culture densities at OD_{600}. The cells were collected by centrifugation at 500 x g for 2 min, the pellets washed twice with 5 ml of ice-cold Hanks balance salt solution and dried under a stream of oxygen - free nitrogen (OFN). Fatty acid methyl esters (FAME) were prepared by incubating the dried yeast cells directly with 1 ml of methylation reagent containing 10 % (v/v) concentrated HCl, 5 % (v/v) 2,2-dimethoxypropane and 85% (v/v) dry methanol for 1 hour at 85^{0}C. After incubation, FAME were extracted by the addition of 1 ml of 1% NaCl solution and 0.5 ml of hexane containing 0.01% butylated hydroxytoluene as antioxidant. The mixture was vigorously mixed and centrifuged at 600 x g for 5 min

to promote phase separation. The top phase was carefully removed and filtered through Whatman No. 1 filter paper into a clean glass test tube, and the solvent evaporated under a stream of OFN. The FAME were purified by thin-layer chromatography and then resuspended in hexane, all as described previously (35). FAME were separated in a Fisons GC8160 gas chromatograph equipped with a chemically bonded CP Wax 52CB fused silica wall coated capillary column (30 m x 0.32 mm i. d., Chrompack U.K. Ltd., London) with an on-column injection system and flame ionization detection. Hydrogen was used as carrier gas with an oven thermal gradient from an initial 50 ºC to 180 ^{o}C at 40 ºC/min, and then to a final temperature of 235 ^{o}C at 2 ºC/min. Individual FAME were identified by comparison with known standards, with a well-characterized fish oil, and by reference to published data, as described previously (35). FAME were quantified using a directly linked PC operating Chrom-Card Software (Thermo-Quest Italia S.P.A., Milan, Italy). All solvents contained 0.01% butylated hydroxytoluene as an antioxidant.

Gas chromatography-Mass Spectrometry (GC-MS). The identities of fatty acids and positions of their double bonds were confirmed by subjecting the picolinyl esters to electron ionization (EI) GC-MS. Free fatty acids were prepared from FAME by alkaline hydrolysis as described by Christie (36). Picolinyl esters were prepared by the method of Balazy and Nies (37). This involves activating the free fatty acid by reaction with 1,1'-carbonyldiimidazole to form the imidazolide, which then reacts with 3-(hydroxymethyl)pyridine under basic conditions to form the picolinyl ester. GC-MS of the picolinyl esters was performed using a Fisons GC8000 gas chromatograph coupled to an MD800 mass spectrometer (Fisons Instruments, Crawley, U.K.). The gas chromatograph was equipped with a fused silica capillary column (60 m x 0.32 mm i.d, 0.25 mm internal film thickness) coated with Zebron ZB-Wax (Phenomenex, Macclesfield, U.K.) and used helium as carrier gas. Samples were applied using on-column injection with the oven temperature programmed to rise from 80 ^{o}C to 250 ^{o}C at 40 ^{o}C per minute.

Materials. Eicosatetraenoic acid (20:4n-3; > 98% pure) was purchased from Cayman Chemical Co., Ann Arbor, U.S.A. Linoleic (18:2n-6), α-linolenic (18:3n-3) and eicosatrienoic (20:3n-6) acids (all >99% pure), BHT, 1,1'-carbonyldiimidazole, 2,2-dimethoxypropane, fatty acid-free BSA, galactose, 3-(hydroxymethyl)pyridine and HBSS, nitrogen base, raffinose, tergitol NP-40 and uracil dropout medium were

obtained from Sigma Chemical Co. Ltd., Dorset, UK. TLC (20- x 20-cm x 0.25-mm) plates precoated with silica gel 60 (without fluorescent indicator) were purchased from Merck, Darmstadt, Germany. All solvents were HPLC grade and were from Rathburn Chemicals, Peebleshire, U.K.

Results

Sequencing revealed that the zebrafish cDNA (Genbank accession number AF309556) comprised 1590 bp, which included an open reading frame specifying a protein of 444 amino acids. The protein sequence included a number of characteristic features of microsomal fatty acid desaturases, including three histidine boxes (Fig. 1). The protein sequence also contained an N-terminal cytochrome b_5 domain containing the heme-binding motif, H-P-G-G, similar to that of other fatty acid desaturases. Further, the third histidine box contained a glutamine residue substituted for the first histidine. The amino acid sequence predicted by the zebrafish ORF indicated that the desaturase candidate possessed 64% identity and 78% similarity to human Δ6 desaturase (Genbank accession number AF126799) and possessed 58% identity and 75% similarity to human Δ5 desaturase (Genbank accession number AF199596). Phylogenetic analysis, comparing a variety of Δ5 and Δ6 desaturases, clustered the zebrafish sequence with mammalian Δ6 desaturase sequences, and with an uncharacterized, putative fish Δ6 desaturase sequence (Fig.2).

The zebrafish desaturase cDNA was functionally characterized by determining the fatty acid profiles of transformed *S. cerevisiae* containing either the pYES vector alone or the vector with the zebrafish cDNA insert (pYESZFB10), grown in the presence of either 18:2n-6, 18:3n-3, 20:3n-6 or 20:4n-3. The fatty acid composition of the yeast transformed with the vector alone showed the four main fatty acids normally found in *S. cerevisiae*, namely 16:0, 16:1n-7, 18:0 and 18:1n-9, together with the four exogenously derived fatty acids (not shown). This is consistent with the fact that *S. cerevisiae* does not possess Δ5 or Δ6 fatty acid desaturase activities. Additional peaks were observed in the profiles of pYESZFB10-transformed yeast grown in the presence of the Δ6 desaturase substrate fatty acids, 18:2n-6 and 18:3n-3, and also in the profiles of pYESZFB10-transformed yeast grown in the presence of the Δ5 desaturase substrate fatty acids, 20:3n-6 and 20:4n-3 (Fig.3A-D). Based on GC retention times, the additional peaks associated with the presence of the zebrafish

cDNA, indicated in Fig. 3A-D, were identified as 18:3n-6, 18:4n-3, 20:4n-6 and 20:5n-3, respectively.

The FAME samples from the transformed yeast incubated with the exogenous PUFA were converted to picolinyl esters and subjected to EI GC-MS to positively identify the structures represented by the additional PUFA peaks produced in cultures containing pYESZFB10. The samples all showed prominent ions at *m/z* = 92, 108, 151 and 164, which are characteristic of picolinyl esters representing fragments about the pyridine ring (Fig.4) (38). The EI spectra of the additional fatty acid in pYESZFB10p-transformed yeast incubated with 18:2n-6 showed a fragmentation pattern with a mass ion of 369 m/z and prominent peaks at 354, 340, 326, 312, 298, 272, 258, 232, 218 and 192 *m/z* (Fig. 4A). The initial interval of 15 (369-354) represented the terminal methyl and was followed by four intervals of 14 indicating four methylene groups. The intervals of 26 (298-272, 258-232 and 218-192) denoted the positions of three double bonds, indicating that this fatty acid is $^{\Delta 12,9,6}$18:3 = 18:3n-6. The EI spectra of the additional fatty acid from cells incubated with 18:3n-3 showed a mass ion of 367 m/z and fragments at 338, 312, 298, 272, 258, 232, 218 and 192 *m/z* confirming this fatty acid as $^{\Delta 15,12,9,6}$18:4 = 18:4n-3 (Fig.4B). The EI spectra of the additional fatty acid produced in cells incubated with 20:3n-6 showed a fragmentation pattern with a mass ion of 395 *m/z* and prominent ions at intervals of 26 (324-298, 284-258, 244-218 and 204-178 *m/z*) denoting the position of the double bonds and indicating that this fatty acid is $^{\Delta 14,11,8,5}$20:4 = 20:4n-6 (Fig.4C). Similarly, the EI spectra of the additional fatty acid in cells incubated with 20:4n-3 showed a fragmentation pattern with a mass ion of 393 *m/z* with prominent ions at intervals of 26 (364-338, 324-298, 284-258, 244-218 and 204-178 m/z) confirming that this fatty acid is $^{\Delta 17,14,11,8,5}$20:5 = 20:5n-3 (Fig.4D). The GC-MS data confirmed that the zebrafish clone is a fatty acid desaturase that introduces double bonds into 18:2n-6 and 18:3n-3 at the Δ6 position, and also into 20:3n-6 and 20:4n-3 at the Δ5 position.

Thus, the analyses revealed that the cells transformed with pYESZFB10 had acquired functional Δ6 and Δ5 fatty acid desaturase activity. Based on the percentages of substrate fatty acids converted to product, the zebrafish gene is more active with Δ6 desaturase substrates than with Δ5 substrates, and preferentially converts n-3 fatty acids rather than n-6 fatty acids (Table 1).

Discussion

The 1590 bp open reading frame of the zebrafish cDNA encodes a protein with substantial similarity to vertebrate Δ6 desaturases. Overall amino acid identities are 64% to human Δ6 desaturase and 58% to human Δ5 desaturase. In addition, the zebrafish protein contains a similar N-terminal cytochrome b_5-like domain and the three catalytically important histidine boxes conserved in all members of the desaturase gene family. It also includes the variant third histidine box that appears to be typical of Δ5 and Δ6 desaturase genes described to-date.

The analyses demonstrated unequivocally that the novel zebrafish cDNA encodes a polypeptide with both Δ5 and Δ6 fatty acid desaturase activities, with slight biases towards n-3 substrates and Δ6 function.

Phylogenetic analysis indicates that, with respect to functionally characterized genes, the zebrafish sequence has highest homology with mammalian Δ6 desaturases, with human Δ5 desaturase appearing to be distinct from the Δ6 desaturase sequences. Human Δ5 and Δ6 desaturase genes (FADS1 and FADS2 respectively) are clustered together with a related gene (FADS3) of unknown function on chromosome 11, and have presumably arisen from a gene duplication event (39). The fact that the Δ5 and FADS3 sequences fall on distinct branches of the phylogenetic tree from that occupied by the Δ6 sequences suggests that this gene duplication event(s) pre-dates the divergence of mammalian and fish evolutionary lines. This suggests that a homologue of the human Δ5 gene should exist in fish species. Interestingly, the nematode *C. elegans* also possesses a cluster of Δ5 and Δ6 desaturase genes that appear to have arisen by gene duplication. However, this duplication clearly occurred after the divergence of nematodes and vertebrates (18,21). Both the mammalian and nematode Δ5 and Δ6 desaturase enzymes have distinct, non-overlapping substrate specificities. It is, therefore, remarkable that the zebrafish desaturase, when expressed in *S. cerevisiae*, exhibits both Δ5 and Δ6 fatty acid desaturase activities with a distinct preference for n-3 compared to n-6 substrates.

Although more fatty acid desaturase genes may be found in both zebrafish and mammals, it is conceivable that the bi-functional desaturase described here is a component of a prototypic vertebrate PUFA biosynthetic pathway that has persisted in a freshwater fish species. That humans and other mammals have two distinct enzymes for Δ5 and Δ6 desaturation may be an adaptation to a terrestrial diet

providing relatively lower amounts of pre-formed C_{20} and C_{22} PUFA than the diets of a vertebrate ancestor that they share with freshwater fish. Functional divergence of the products of a putative ancient gene duplication event is a possible mechanism underlying adaptation to such a dietary change.

Conversely, the apparent deficiencies in the fatty acid desaturation pathway in some marine fish species, many of which are strictly piscivorous, may be a result of relaxation of constraints on a prototypic pathway in an environment providing a diet that is naturally rich in C_{20} and C_{22} PUFA. Certainly, the discovery of a bifunctional enzyme in zebrafish is consistent with findings in the piscivorous marine species, turbot (*Scophthalmus maximus*). In turbot, a deficiency in the fatty acid desaturation/elongation pathway has been ascribed to lack of $C_{18\text{-}20}$ elongase function, while both Δ6 and Δ5 desaturase capabilities have been retained (40). However, in another marine species, gilthead sea bream (*Sparus aurata*), a block in the fatty acid desaturation/elongation pathway has been identified as a deficiency in Δ5 desaturase activity (41). In this case it is possible that an homologous desaturase enzyme has lost significant Δ5 desaturase activity, thus limiting the efficiency of the PUFA biosynthetic pathway in gilthead sea bream. Studies of the desaturase genes of a range of fish species are ongoing with the purpose of examining these possibilities.

The ability of fish to provide 20:5n-3 and 22:6n-3 for human and other animal diets has become an increasingly important subject in recent years following the global stagnation and decline of capture marine fisheries (42). These have hitherto been the major source of 20:5n-3 and 22:6n-3 in human and animal diets (13). Increased fish farming can potentially offset the effects of this decline in a key marine resource. However, where farmed marine species are concerned, a dietary source of 20:5n-3 and 22:6n-3 is required, and this has hitherto been provided by fish oils from captured marine "feed species" such as sand eels, capelin and anchovies (42). Such "feed species" are themselves finite resources which, in the case of anchovies, is markedly reduced by climatic phenomena such as El Nino. Understanding the molecular basis of the differences between marine and freshwater fish in their abilities to synthesize 20:5n-3 and 22:6n-3 may suggest ways in which the reliance of farmed marine species on marine fish oils could be reduced.

Here we report for the first time a fatty acid desaturase with both Δ6 and Δ5 activities. Such an enzyme could contribute to a biotechnological solution for 20:5n-3

and 22:6n-3 production, ultimately relieving pressure for unsustainable extraction of these key nutrients from natural marine sources.

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Legends to Figures:

Figure 1. Alignment of the predicted amino acid sequences of the zebrafish desaturase (DRD5/6) and human Δ5 (HSFADS1) and Δ6 desaturases (HSFADS2). The three ORFs encode 444 amino acid residues. Identical amino acids are in black, the cytochrome b_5-like domain is underlined by asterisks and the three histidine-rich domains are underlined.

*Figure 2***.** Phylogeny of desaturase deduced amino acid sequences. Sequences marked with an asterisk are not functionally characterized. Data base accession numbers for the nucleic acid sequences are indicated. Conditions used for neighbor joining tree construction are described in the text and were applied using ClustalW and NJplot. Horizontal branch lengths are proportional to the number of amino acid replacements per position, the scale bar indicating this value. Numbers represent the percentage frequencies with which the tree topology presented here was replicated after 1000 bootstrap iterations.

Figure3. Identification of fatty acid desaturation products in transgenic yeast. FAMEs were extracted from yeast transformed with pYESZFB10 grown in the presence of either **A**: 18:2n-6, **B**: 18:3n-3, **C**: 20:3n-6, and **D**: 20:4n-3. The first four peaks in panels A-D are 16:0, 16:1n-7, 18:0 and 18:1n-9, respectively. The fifth peaks in each panel are the exogenously added fatty acids 18:2n-6 (A), 18:3n-3 (B), 20:3n-6 (C), and 20:4n-3 (D), respectively. The sixth peaks in each panel, arrowed, were tentatively identified (based on retention times) as 18:3n-6 (A), 18:4n-3 (B), 20:4n-6 (C) and 20:5n-3 (D), respectively.

Figure 4. Mass spectra of the arrowed novel peaks in Figure 3. Picolinyl esters were prepared from FAME extracted from yeast transformed with pYESZFB10 grown in the presence of either **A**: 18:2n-6, **B**: 18:3n-3, **C**: 20:3n-6, and **D**: 20:4n-3, and analyzed by GC-MS as described in the Methods section. The identities of the novel peaks were confirmed as **A**: 18:3n-6, **B**: 18:5n-3, **C**: 20:4n-6, **D**: 20:5n-3.

Table 1. pYESZFB10 Substrate conversion (%) and activity type

Substrate fatty acid	Substrate conversion	Desaturase activity
18:2n-6	11.7	Δ6
18:3n-3	29.4	Δ6
20:3n-6	8.3	Δ5
20:4n-3	20.4	Δ5

Fig.1.

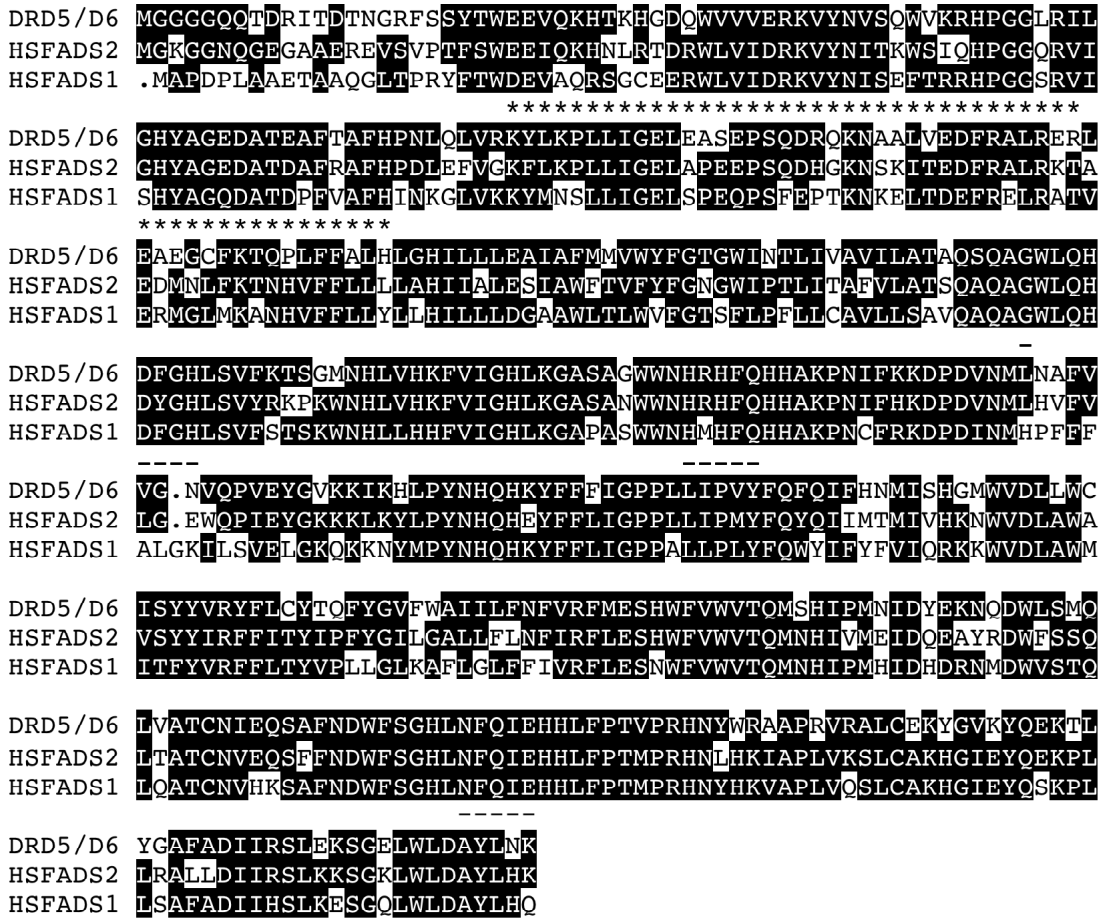

DRD5/D6 MGGGGQQTDRITDTNGRFSSYTWEEVQKHTKHGDQWVVVERKVYNVSQWVKRHPGGLRIL
HSFADS2 MGKGGNQGEGAAEREVSVPTFSWEEIQKHNLRTDRWLVIDRKVYNITKWSIQHPGGQRVI
HSFADS1 .MAPDPLAAETAAQGLTPRYFTWDEVAQRSGCEERWLVIDRKVYNISEFTRRHPGGSRVI
DRD5/D6 GHYAGEDATEAFTAFHPNLQLVRKYLKPLLIGELEASEPSQDRQKNAALVEDFRALRERL
HSFADS2 GHYAGEDATDAFRAFHPDLEFVGKFLKPLLIGELAPEEPSQDHGKNSKITEDFRALRKTA
HSFADS1 SHYAGQDATDPFVAFHINKGLVKKYMNSLLIGELSPEQPSFEPTKNKELTDEFRELRATV
DRD5/D6 EAEGCFKTQPLFFALHLGHILLLEAIAFMMVWYFGTGWINTLIVAVILATAQSQAGWLQH
HSFADS2 EDMNLFKTNHVFFLLLLAHIIALESIAWFTVFYFGNGWIPTLITAFVLATSQAQAGWLQH
HSFADS1 ERMGLMKANHVFFLLYLLHILLLDGAAWLTLWVFGTSFLPFLLCAVLLSAVQAQAGWLQH
DRD5/D6 DFGHLSVFKTSGMNHLVHKFVIGHLKGASAGWWNHRHFQHHAKPNIFKKDPDVNMLNAFV
HSFADS2 DYGHLSVYRKPKWNHLVHKFVIGHLKGASANWWNHRHFQHHAKPNIFHKDPDVNMLHVFV
HSFADS1 DFGHLSVFSTSKWNHLLHHFVIGHLKGAPASWWNHMHFQHHAKPNCFRKDPDINMHPFFF
DRD5/D6 VG.NVQPVEYGVKKIKHLPYNHQHKYFFFIGPPLLIPVYFQFQIFHNMISHGMWVDLLWC
HSFADS2 LG.EWQPIEYGKKKLKYLPYNHQHEYFFLIGPPLLIPMYFQYQIIMTMIVHKNWVDLAWA
HSFADS1 ALGKILSVELGKQKKNYMPYNHQHKYFFLIGPPALLPLYFQWYIFYFVIQRKKWVDLAWM
DRD5/D6 ISYYVRYFLCYTQFYGVFWAIILFNFVRFMESHWFVWVTQMSHIPMNIDYEKNQDWLSMQ
HSFADS2 VSYYIRFFITYIPFYGILGALLFLNFIRFLESHWFVWVTQMNHIVMEIDQEAYRDWFSSQ
HSFADS1 ITFYVRFFLTYVPLLGLKAFLGLFFIVRFLESNWFVWVTQMNHIPMHIDHDRNMDWVSTQ
DRD5/D6 LVATCNIEQSAFNDWFSGHLNFQIEHHLFPTVPRHNYWRAAPRVRALCEKYGVKYQEKTL
HSFADS2 LTATCNVEQSFFNDWFSGHLNFQIEHHLFPTMPRHNLHKIAPLVKSLCAKHGIEYQEKPL
HSFADS1 LQATCNVHKSAFNDWFSGHLNFQIEHHLFPTMPRHNYHKVAPLVQSLCAKHGIEYQSKPL
DRD5/D6 YGAFADIIRSLEKSGELWLDAYLNK
HSFADS2 LRALLDIIRSLKKSGKLWLDAYLHK
HSFADS1 LSAFADIIHSLKESGQLWLDAYLHQ

Fig.2

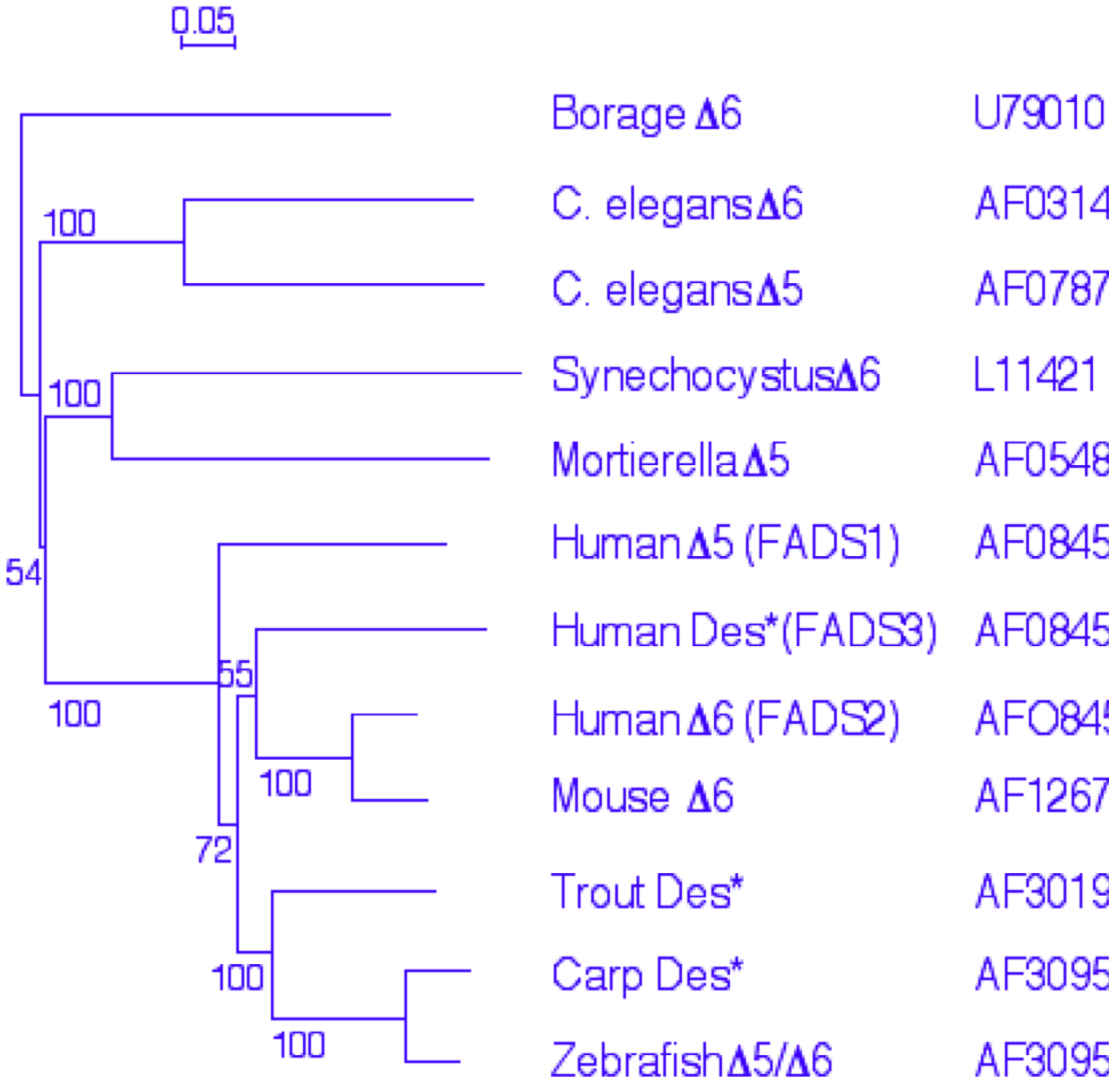

0.05
Borage Δ6
U79010
C. elegans Δ6
AF0314
C. elegans Δ5
AF0787
Synechocystus Δ6
L11421
Mortierella Δ5
AF0548
Human Δ5 (FADS1)
AF0845
Human Des*(FADS3)
AF0845
Human Δ6 (FADS2)
AFO84
Mouse Δ6
AF1267
Trout Des*
AF3019
Carp Des*
AF3095
Zebrafish Δ5/Δ6
AF3095
100
100
54
55
100
100
72
100
100

Fig.3.

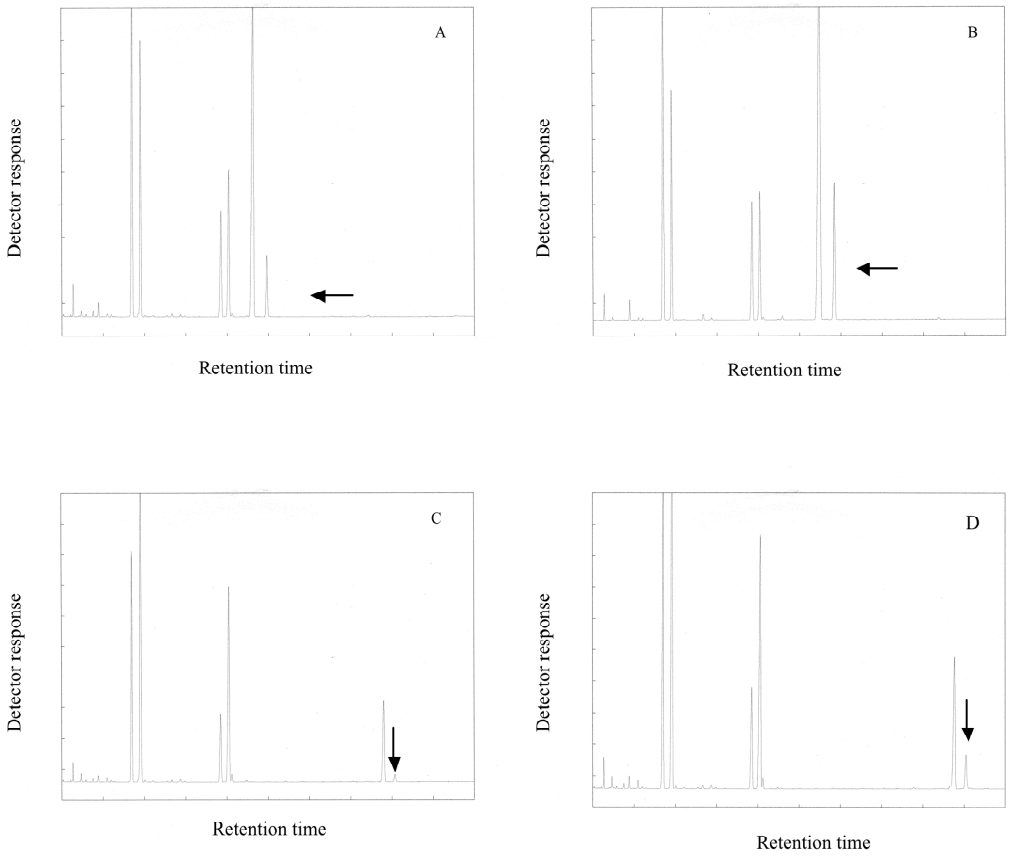
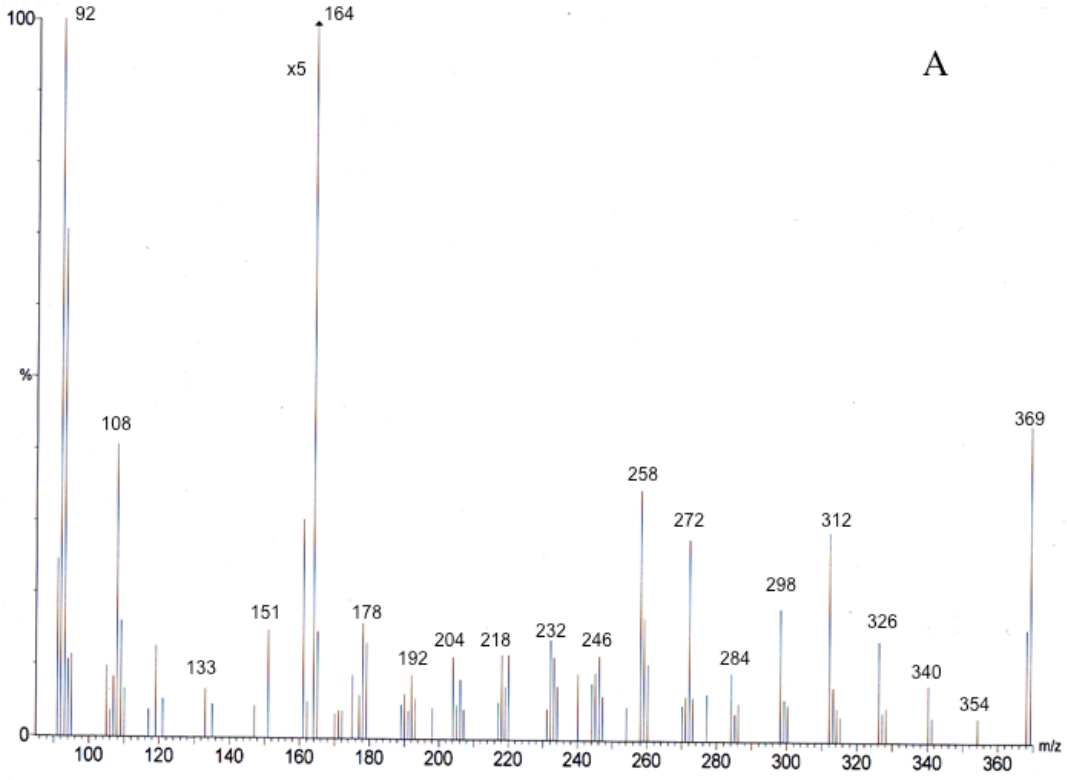

A
Detector response
Retention time
B
Detector response
Retention time
C
Detector response
Retention time
D
Detector response
Retention time

Fig.4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218
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258
272
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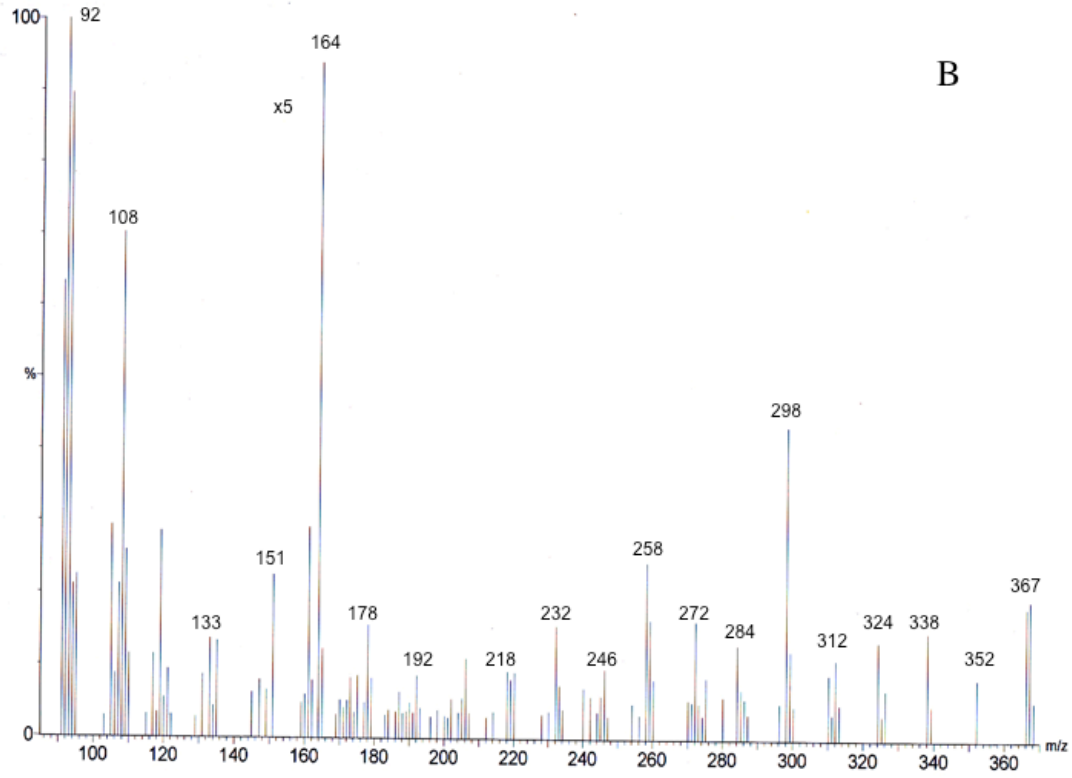
240
260
280
300
320
340
360
m/z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%
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164
x5
108
151
133
178
192
218
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246
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272
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260
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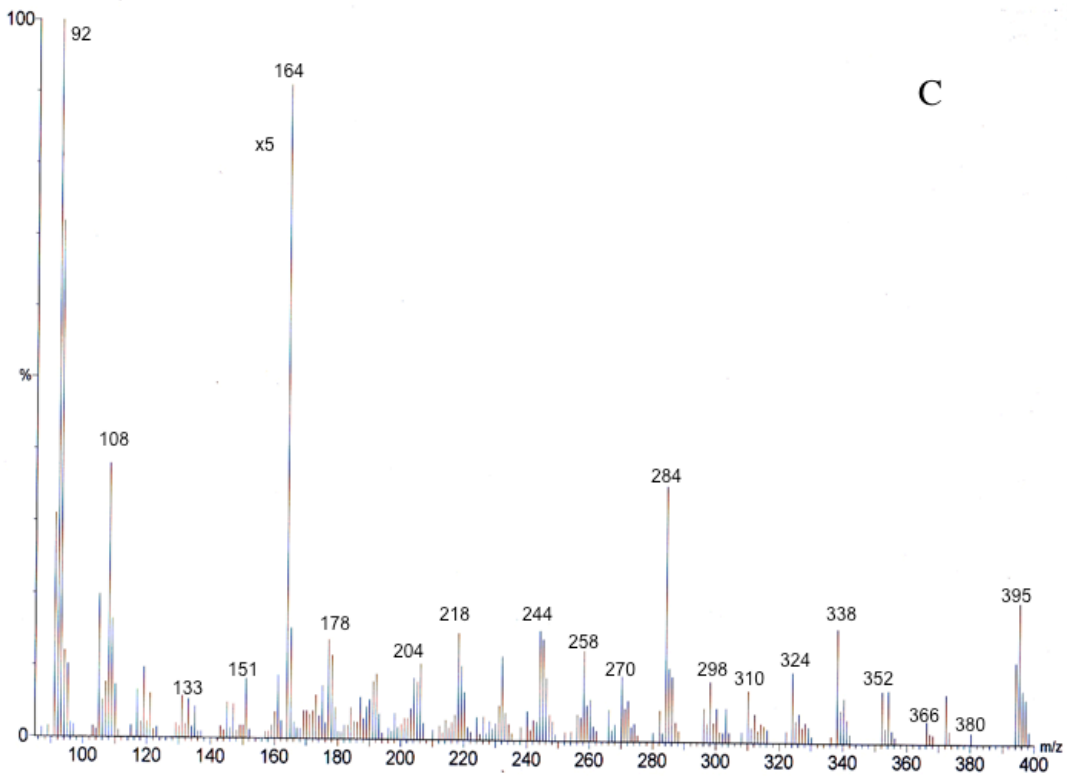
340
360
m/z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352
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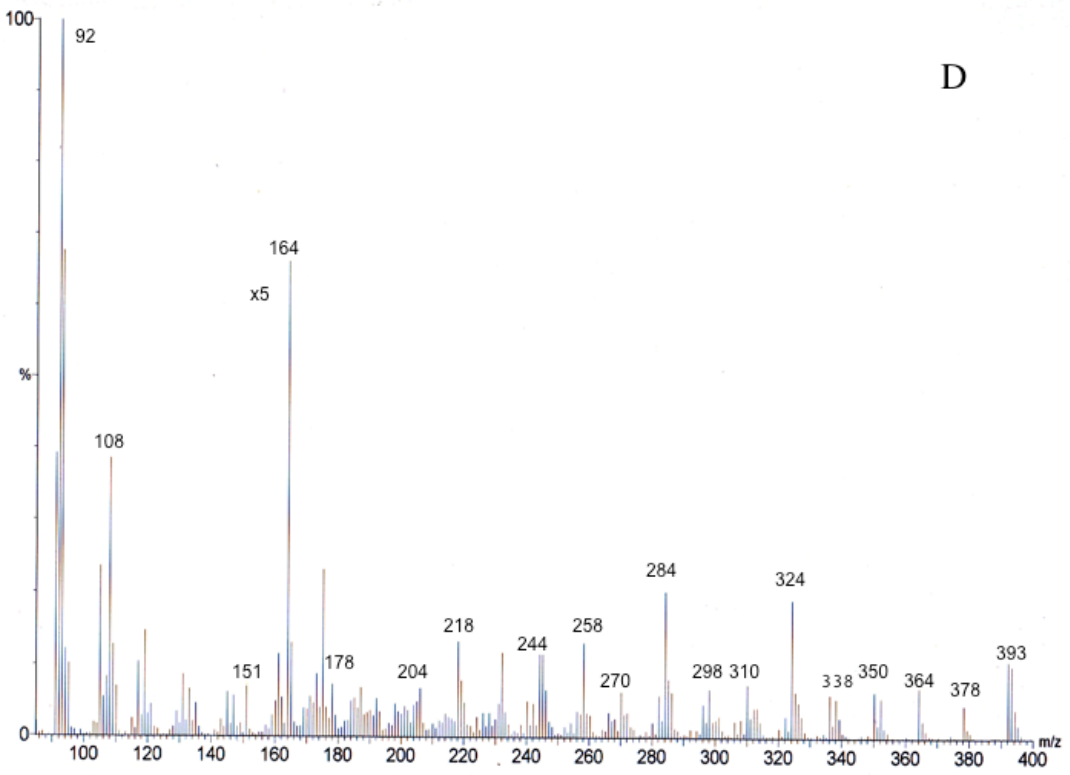
m/z


D
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164
x5
%
108
151
178
204
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258
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298
310
324
338
350
364
378
393
0
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
m/z